Starch composites with aconitic acid

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Abstract

The aim of this project is to examine the effectiveness of using aconitic acid (AcA), a tricarboxylic acid which contains a carbon/carbon double bond (C=C), to enhance the properties of starch-based films. Starch/glycerol cast films were prepared with 0, 2, 5, 10 and 15 wt% AcA (starch wt% basis) and the properties analysed. It was shown that AcA acted as both a cross-linking agent and also a strong plasticising agent. The 5 wt% AcA derived starch films were the most effectively cross-linked having the lowest solubility (28 wt%) and decreased swelling coefficient (35 vol.%) by approximately 3 times and 2.4 times respectively compared to the control film submerged in water (23 °C). There was also a significant increase in the film elongation at break by approximately 35 times (compared to the control) with the addition of 15 wt% AcA, emphasising the plasticising effect of AcA. However, generally there was a reduced tensile strength, softening of the film, and reduced thermal stability with increased amounts of AcA.

Keywords Aconitic Acid; Cross-link; Starch; Solubility; Elastomer.

Chemical compounds studied in this article

Aconitic acid (PubChem CID: 444212); Starch (PubChem CID: 24836924); Glycerol (PubChem CID: 753).

Highlights

- Improved insight and knowledge of the dual role of aconitic acid as a plasticising and a cross-linking agent.
- The addition of aconitic acid to starch film impacts upon the elastomeric properties.
- Reduced water solubility (maintain flexibility) of starch/solvent-casting films with aconitic acid.

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1. INTRODUCTION

Cross-linking is a way of improving the properties of starch-based film, with improved mechanical properties and resistance to solubility. Many of the chemicals used for cross-linking starch are relatively toxic, expensive and/or do not produce improved properties (Ghanbarzadeh *et al.*, 2011; Reddy & Yang, 2010). Citric acid (CA), tartaric acid (TA) and malic acid (MA) are typically inexpensive and non-toxic organic acids used to cross-link

starch (Ghanbarzadeh *et al.*, 2011; Ma *et al.*, 2009; Olivato *et al.*, 2012; Reddy & Yang, 2010; Shi *et al.*, 2008; Shi *et al.*, 2007; Stewart, 2007). Other agents that give food grade cross-linked starches include sodium trimetaphosphate (STMP), sodium tripolyphosphate (STPP), epichlorohydrin (EPI) and phosphoryl chloride ($POCl_3$) (Kaur *et al.*, 2006; Koo *et al.*, 2010; O'Brien *et al.*, 2009; Polnaya *et al.*, 2013).

During film formation the presence of a cross-linking agent assists in the creation of a network of bonded cross-links within the film. These crosslinks generally improve the film moisture resistance since the network helps to prevent film swelling, which in turn reduces moisture absorption. Cross-linking also helps prevent excessive crystallisation (retrogradation) that contributes to film brittleness, cracking and/or fracturing by restricting the mobility of the starch polymer chains, inhibiting them from forming crystalline polymorph structures.

Ghanbarzadeh *et al.* (2011) using the solvent cast method, chemically modified starch/glycerol films by cross linking the starch polymer with CA to produce a more moisture resistant and stronger film. The water vapour barrier property and tensile strength were improved significantly ($p < 0.05$) as the CA percentage increased from 0 to 10 wt% (based on starch wt).

Reddy and Yang (2010) also used CA as a cross-linking agent in cast starch/glycerol films. The use of 5 wt% CA in combination with 15 wt% glycerol increased the film strength by 150% (after thermal curing). Water solubility tests showed that cross-linking occurred in the CA derived films, since these films only lost 25 wt%, whereas non-cross-linked film (without CA) lost 75 wt%. Other similar tricarboxylic organic acids (e.g. Ma and TA) also act as starch cross-linking agents (Ghanbarzadeh *et al.*, 2011; Olivato *et al.*, 2012; Reddy & Yang, 2010). However, the use of a tricarboxylic acid containing a carbon/carbon double bond (C=C) such as AcA (Fig. 1) may enhance the cross-linking process compared to those without it.

AcA is found to be naturally present in molasses, which it may be isolated from (Gil, 2007; Kanitkar *et al.*, 2013; McCalip & Seibert, 1941). The use of AcA as a cross-linking agent is desirable since it originates from a renewable plant source (molasses) and also is not toxic as it is approved by the Food and Drug Agency (FDA) as a food flavouring additive (*Select Comittee on Generally Recognised as Safe Subatances (SCOGS) Opinion: Aconitic Acid*, 1974).

In this project the effectiveness of aconitic acid (AcA) to enhance the properties of starch-based films was assessed. Starch/glycerol cast films were prepared with 0, 2, 5, 10 and 15 wt% AcA (starch wt% basis) and the film properties assessed included, moisture uptake, crystallinity and solubility/swelling besides thermal and mechanical tensile testing. The changes in properties were explained by the interactions and changes in the molecular structure of the films.

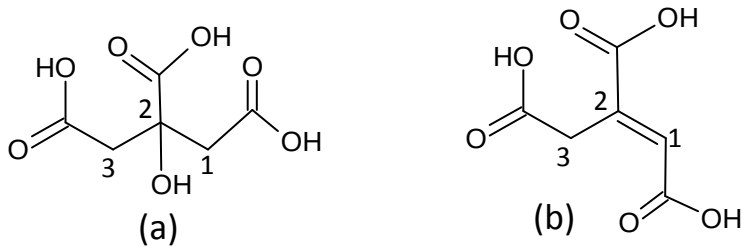

Fig. 1. Molecular structures of: (a) Citric acid and (b) trans-aconitic acid.

2. MATERIALS AND METHODS

2.1 Materials

The starch type used was a potato derived starch (25% amylose content) brand named 'Soluble starch' as well as potassium bromide (KBr) was purchased from Sigma-Aldrich (St. Louis, MO, USA). The plasticiser, glycerol, was purchased from Merck (Darmstadt,

Germany) and was added to the starch to produce the film. Trans-aconitic acid (99.9% Reagent) was supplied by the Nanjing Fayekong Chemcial Co.,Ltd, China.

2.2 Film preparation method

Mixtures of Soluble (Sigma brand name) (potato) starch (15 g), glycerol (3 g) and distilled water (500 mL) along with (trans) AcA in the amounts of 0, 0.3, 0.75, 1.5 and 2.25 g for 0, 2, 5, 10 and 15 wt% films respectively (based on starch wt) were prepared. All solutions were boiled for a total of 30 minutes ensured complete starch gelatinisation. The films were dried overnight in a 65 °C oven. All films were subsequently conditioned at 58% RH (23±1 °C) in a humidity chamber before assessing the film properties.

2.3 Moisture and conditioning

All films were placed into a relative humidity (RH) chamber that contained a saturated aqueous salt solution of NaBr which created a 58% RH atmosphere at 23±1 °C (Greenspan, 1976). The films reached moisture equilibrium (i.e. constant wt) in approximately 5 to 7 days. Moisture content (M_C) was determined by drying the films in an oven at 105 °C to a constant weight. The weight loss was used to calculate M_{eq} wt% on a dry basis (Eq. 1):

$$M_{eq} = \frac{M_i - M_d}{M_i} \times 100\% \qquad \text{Equation 1}$$

where M_i and M_d are the mass of initial and dried samples, respectively. Moisture uptake was determined based on the average of four specimens.

2.4 X-ray diffraction and crystallinity

X-ray diffraction (XRD) was performed by a diffractometer (XPERT-PRO) using Cu-$K_{\alpha 1}$ radiation (λ = 1.5406 Å) and Cu-$K_{\alpha 2}$ radiation (λ = 1.5444 Å) at 40 kV and 40 mA with a divergent slit fixed at 0.5° and a 0.5 mm receiving slit, without using any monochomator. The

scanning angle range was from 4° to 30° (2θ) with a step size of 0.0167° (2θ). Wide angle X-ray diffractograms of the starch films were made by exposing the film samples cut into approximately 15 mm × 20 mm rectangular shapes to the X-ray beam. The degree of bulk film crystallinity (X_c) was determined using the method described by (Dai *et al.*, 2008) (Equ. 2):

$$X_c = A_c / \left(A_c + A_a \right) \qquad \text{Equation 2}$$

where A_c refers to the sum of the crystallised peak areas above the amorphous area and A_a refers to the amorphous area on the X-ray diffractogram.

2.5 Thermogravimetric analysis

Thermogravimetric analysis (TGA) and differential thermal gravimetric (DTG) analyses of (4.5 mg) samples were performed using a TA instrument model Q500 (New Castle, DE 19720, USA) at a heating rate of 5°C /min from ambient temperature to 800°C. A nitrogen atmosphere with a sample flow rate of 60 mL/min and balance flow rate of 40 mL/min was used to avoid the production of unwanted products in the presence of oxygen during analysis (Rodriguez *et al.*, 2010). The estimate of the standard deviation (absolute) for the TGA technique, based on duplicate experiments (10 degrees of freedom) is 0.22%.

2.6 Tensile Testing

The tensile strength, Young's modulus, stress at break and stress at yield were measured using an Instron 4505 tensile testing machine (High Wycombe, UK) equipped with a 100 N load cell. Test specimens were cut from the (approximately 0.25 mm thick) cast films into a rectangular shape of 7.0 mm wide by 50 mm long. A constant rate of strain of 10 mm/min was applied to the specimen securely held between grips (ASTM, 2008). Soft grips were employed to help prevent the delicate starch polymer specimens from being crushed or damaged by the machine grips. The data was averaged over 5 to 8 specimens to give the final result.

2.7 Dynamic mechanical thermal analysis

Dynamic mechanical analyses (DMTA) was performed on a DMTA-IV from Rheometric Scientific in the tensile mode. The width and the length of the samples were 5.6 mm and 25 mm respectively. Samples were clamped at each end in the machine, and tested in the tensile mode at a constant strain rate of 0.01 % and a frequency of 1 Hz. The range of temperature scanned was approximately from -70 to 100°C at a heating rate of 2 °C/min. Liquid nitrogen was used for cooling the samples. A thin coating of petroleum jelly was applied to the outside of the films to avoid water evaporation and impact on the glass transition temperature (T_g) values.

2.8 Fourier transform-Infrared spectroscopy

Infrared spectra were collected using a Nicolet 870 Nexus Fourier transform infrared (FTIR) spectrometer equipped with an attenuated total reflection (ATR) accessory (Madison, WI, USA). Using this system, small pieces of polymer film were brought directly into contact with the ATR objective diamond crystal using slight pressure in order to collect the spectrum. Absorption sspectra were collected in the range from 4000 to 550 cm^{-1}, using 64 scans at a resolution of 4 cm^{-1} while corrected against the background spectrum. Spectra were manipulated and plotted with the use of the Galactic 187 Industries Corporation GRAMS/32 software package (Salem, NH, USA). The final FTIR spectra were normalised with respect to the distinct starch peak located at 1150 cm^{-1} since this peak was the least changed among the FTIR spectra for the composites.

2.9 Swelling and solubility analysis

The swelling studies are one of the ways to determine the degree of cross-linking in films (Radi *et al.*, 2010). The initial mass of the conditioned film (58% RH 25 °C) samples (W_{init}) was measured and the density (ρ_{init}) determined based on the moisture content to give the initial film

volume ($V_{init} = W_{init} / \rho_{init}$). Film samples were directly submerged into a flask containing liquid distilled water at 23±1 °C (ambient conditions) that was decanted off and collected each day as the water soluble components and uncross-linked polymers dissolved into solution. The collected solution was replaced each day with an equivalent amount of distilled water. The hydrated film gel was removed and excess water carefully blotted off the surface with tissue paper before being weighed to give the film gel weight (W_{gel}). The procedure of decanting, collecting, replacing the water and weighing the film gel was repeated until the gel weight remained constant indicating swelling equilibrium had been reached (after 4 days). The hydrogels were dried in an oven at 40 °C and the gel dry weight ($W_{dry \cdot gel}$) measured. The dry gel films were then conditioned (58% RH at 23 °C) and weighed giving the moisture and density of the conditioned gel film. The density of the swollen gel (ρ_{gel}) was then determined and hence the film gel volume ($V_{gel} = W_{gel} / \rho_{gel}$). The ρ_{gel} for 0, 2, 5, 10 and 15 wt% AcA derived film was determined to be approximately 1.004, 1.023, 1.016, 1.008 and 1.007 g/mL respectively, slightly higher than for water 0.9975 g/mL (23 °C) (Perry & Green, 2008). The collected soluble water fractions were oven dried (40 °C) to constant weight and the dry soluble component weight ($W_{dry \cdot sol}$) determined.

The volume fraction (Q) was determined by the ratio of the swollen gel volume (V_{gel}) over the initial conditioned volume (V_{init}) (Eq. 3) (Elbert *et al.*, 2001).

$$Q = \frac{V_{gel}}{V_{init}} \qquad \text{Equation 3}$$

The soluble fraction was determined by the ratio of $W_{dry \cdot sol}$ from the solution water over the sum of $W_{dry \cdot gel}$ and $W_{dry \cdot sol}$ (total weight) (Eq. 4).

$$Sol\% = \frac{W_{dry \cdot sol}}{W_{dry \cdot gel} + W_{dry \cdot sol}} \times 100\% \qquad \text{Equation 4}$$

2.10 Average molecular weight between cross-links (M_c)

The swelling of the film at equilibrium in water is the basis for estimating the average molecular weight between cross-linked polymer network structures (M_c) using the Flory-Rehner rubber elasticity model equation (Eq. 5) (Elbert *et al.*, 2001; Flory, 1953; Flory & Rehner, 1943; Radi *et al.*, 2010):

$$\frac{1}{Mc} = \frac{2}{M_n} - \frac{\frac{(1/\rho_{poly})}{V_{sol}}\left[\ln(1-\phi)+\phi+\chi\phi^2\right]}{\left[\phi^{1/3}-\frac{\phi}{2}\right]} \qquad \text{Equation 5}$$

where ϕ is the volume fraction of polymer in the swollen gel ($\phi = \frac{V_{init}}{V_{gel}} = \frac{1}{Q}$) (or reciprocal of the "swelling volume") ρ_{poly} is the initially conditioned polymer film density before hydration (the specific polymer volume $1/\rho_{poly}$), χ is the polymer solvent interaction parameter (0.48 for starch/water) (Habeych *et al.*, 2009), V_{sol} the molar volume of water (18.060 ml/gmole) and M_n the number average molecular weight of starch polymer chains before cross-linking (1.350 x 10^6 g/mole) (Lisinska & Leszczynski, 1989).

To calculate the volume fractions of the hydrogels the density of potato starch, density of water and density of glycerol were assumed to be 1.53 g/mL (DeLeo *et al.*, 2009), 0.998 g/mL (Perry & Green, 2008) and 1.26 g/mL (Merk Pty. Ltd) respectively.

2.11 Statistical analysis

Analysis of variance (ANOVA) and tests of significance were performed with Minitab® version 16.1.0 (State College, PA, USA) using a confidence level of 95% ($p<0.05$) to detect differences among mean values of films properties. Results are expressed as mean values.

3. Results for aconitic acid derived films

3.1 Moisture and crystallinity

The moisture of the AcA derived films marginally increased as the AcA content increased (Table 1) that may be due to the free residual AcA left in the film acting as a plasticiser. In fact Ghanbarzadeh *et al.* (2011) found a similar effect for starch film with increased CA content. Ma *et al.* (2009) also encountered film plasticizing when the CA content exceeded 9 wt%. It was reported that increasing CA content causes the increase in the interchain spaces and chain mobility due to inclusion of residual free CA molecules between the polymer chains which in turn promotes water absorption (Ghanbarzadeh *et al.*, 2011). It is assumed that AcA now behaved in the same manner and reduced hydrogen bonding between starch polymer chains which softens the film. However, if the hydrogen bonding between AcA and starch molecules is stronger than that of the glycerol, then glycerol becomes displaced, with increased phase separation in the starch matrix. This may cause increased moisture absorption, as in the case of increased glycerol content. However, the strong bonding between AcA and starch hydroxyl groups (similarly to CA) may prevent water from hydrogen bonding with the starch preventing increased moisture uptake with increased AcA content (Reddy & Yang, 2010; Yu *et al.*, 2005). Furthermore, the cross-linking by AcA reacting with the starch matrix and forming a three dimensional network will prevent film swelling and limit moisture absorption. However, it should be noted that the moisture content remained relatively constant from 5 to 15 wt% AcA films. Moisture increase may lead to increased crystallinity and film brittleness. However cross-linking the starch polymer chains restricts their movement helping to preventing crystal formation.

Table 1 Moisture and crystallinity properties of film*

AcA (%)	Moisture (%)	Crystallinity (%)
0.00	7.0b	1.02d
2.00	7.5b	2.82a
5.00	8.3ab	1.67c
10.00	8.6a	2.29b
15.00	8.8a	3.22a

* Figures under each property with the same letters are not significantly different ($P < 0.05$).

The crystallinity of AcA derived film does not show a trend with increasing AcA content. It did show, however, that the film crystallinity increased with the addition of AcA (Table 1). This crystallinity increase indicates that although AcA strongly hydrogen bonds with the hydroxyl starch polymer groups there develops increased areas of glycerol phase separation in the starch matrix allowing limited moisture absorption and increased freedom of movement (free volume) for the starch polymer chains allowing them to crystallise.

The X-ray diffraction patterns of the AcA derived films (Fig. 2) reveals an increase in the profile of the prominent B type crystallinity main peak (typical for potato starch) located at 17° (2θ) (van Soest *et al.*, 1996; van Soest *et al.*, 1995) with the increase of AcA (Table 1).

Crystallinity is affected by the glass transition temperature (T_g) that indicates when the film changes from a 'glassy' state to a softer more pliable state at temperatures above the T_g (Table 4). The film is in the 'glassy' state at temperatures below the T_g and polymer chain movements are increasingly restricted by bonding interactions between the polymer chains that are inhibited from forming crystal structures.

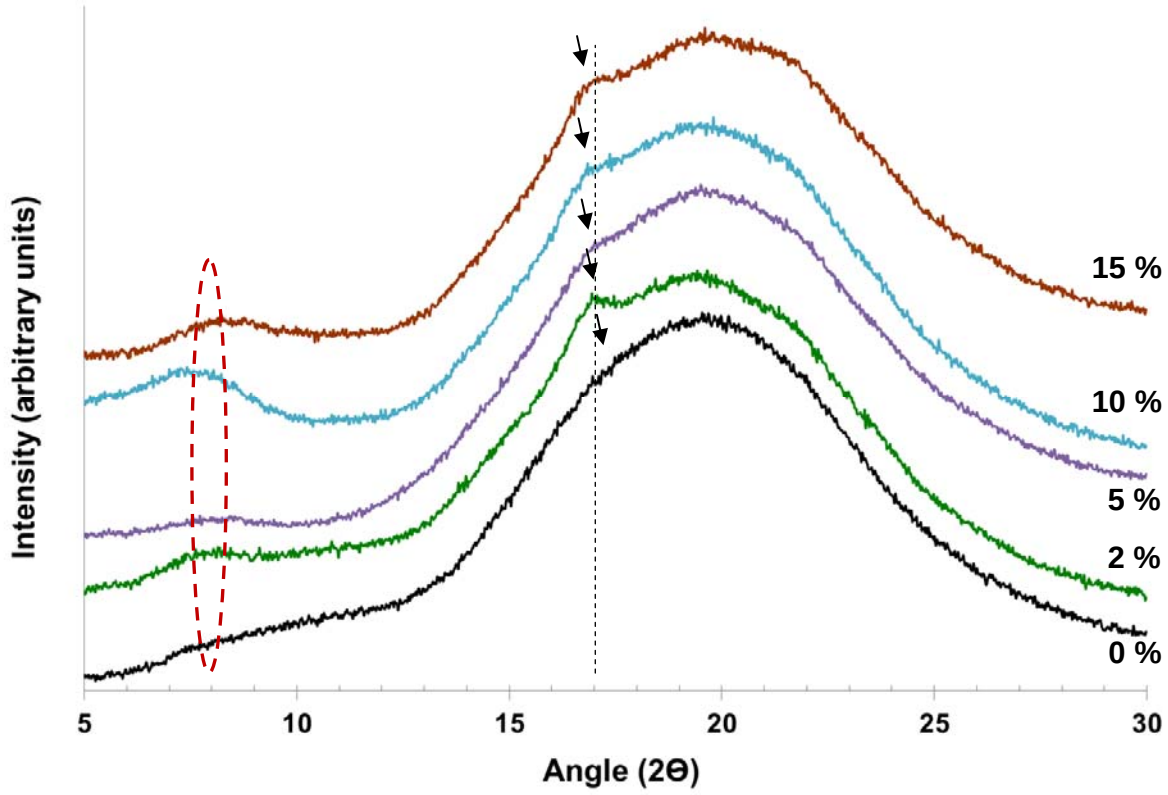

Fig. 2. **X-ray diffraction patterns of cast films (conditioned at 23 °C, 58% RH) with each film derived from starch/glycerol mixtures that contain: 0, 2, 5, 10 and 15 wt% aconitic acid (starch wt% basis) from bottom to top.**

Interestingly the 2 and 15 wt% AcA derived films both show similarly the greatest 17° (2θ) peak intensity and crystallinity (~3%). The reason for this may be related to these films having similar and the lowest T_g (~50°C) values for AcA derived films (Table 4). However, although the T_g values are still well above ambient temperature (23 °C) and the films being in the 'glassy' state, are more likely to crystallise than the other films which have higher T_g values. The control film has the highest T_g (99 °C) and lowest crystallinity (1%) as expected since it has the least plasticiser (moisture and AcA) content and contains more tightly bonded starch polymer chains.

It is also observed that the film structure becomes more amorphous with increases in the derived AcA film content as indicated by the prominent amorphous halo (Nakamura *et al.*, 1989) or 'rounded hump' located in the 8° (2θ) region in most of the samples derived from

AcA (Fig. 2) This may be due to the presence of AcA rearranging the film structure and creating distinct regional phases. It was noted an increase in glycerol phase separation occurrences with increases in the AcA content used for deriving film that may contribute towards an increased plasticising effect.

3.2 Thermal gravimetric analysis (TGA)

The film thermal degradation is indicated by the thermogravimetric (TG) and derivative thermogravimetric (DTG) graphs (Figs. 3 and 4). Thermal degradation temperatures generally decrease with an increase in AcA for the derived films as indicated by: the onset degradation temperature at 5wt% dry mass loss (T_o), the temperature at maximum rate of mass loss (T_{max}) and the temperature at 50 wt% dry mass loss (T_{50}) (Table 2). There is also an increase in the number of the DTG lower temperature side peaks (Pk_1, Pk_2 and Pk_3) as part of the main degradation peak, associated with AcA derived films (Figs. 3 and 4).

The initial weight loss maximum (DTG peak) occurs approximately at 100 °C as volatiles are driven off considered mostly to be water since glycerol vaporises/degrades at higher temperatures (199 to 249 °C) and the initial weight loss is similar to the film moisture content (Castelló *et al.*, 2009; Shi *et al.*, 2007; Yu *et al.*, 2005).

Table 2 Thermal degradation properties of aconitic acid derived film*

AcA (%)	T_o (°C)	T_{max} (°C)	T_{50} (°C)	Pk_1** (°C)	Pk_2** (°C)	Pk_3** (°C)
0	257a	311b	310b	---	---	---
2	258a	315a	321a	263b	---	---
5	245b	299c	307c	250c	272b	---
10	239c	247d	302d	276a	283a	---
15	237c	263e	303d	224d	255c	278

* Figures under each property with the same letters are not significantly different ($P < 0.05$). **Degradation side Peaks (Pk_1, Pk_2 and Pk_3) as shown on DTG graphs at increasing temperatures.

Thermal degradation for the 0 wt% AcA derived (control) starch/glycerol film is a one step process (after moisture loss) with only one main degradation (DMT) peak (Figure 3 and 4) (Gilfillan *et al.*, 2012; Shi *et al.*, 2007). However, there is a two step degradation process for all derived films containing AcA, in a similar way to that of CA derived films by Shi *et al.* (2007). The first degradation weight loss DTG peak (after moisture loss) may be due to: decomposition of residual AcA; acid hydrolysis of starch by AcA; or the cross-link formation reaction of AcA with the starch polymer chains (Ma *et al.*, 2009; Shi *et al.*, 2007; Yu *et al.*, 2005). The second major weight loss DTG peak is due to degradation of the starch polymer complex.

In general T_o and T_{max} decrease from 257 to 237 °C and 311 to 263 °C respectively for 0 to 15 wt% additions of AcA derived films. Similar results were obtained for CA derived films by Ma *et al*. (2009) and Shi *et al*. (2007). The reduction in the major weight loss peak T_{max} in AcA derived films may be considered in a similar way to that of CA derived films. Ma *et al.* (2009) considered the reduction in T_{max} may be attributable to the ester bonds formed during the cross-linking reaction that break at lower temperatures of degradation.

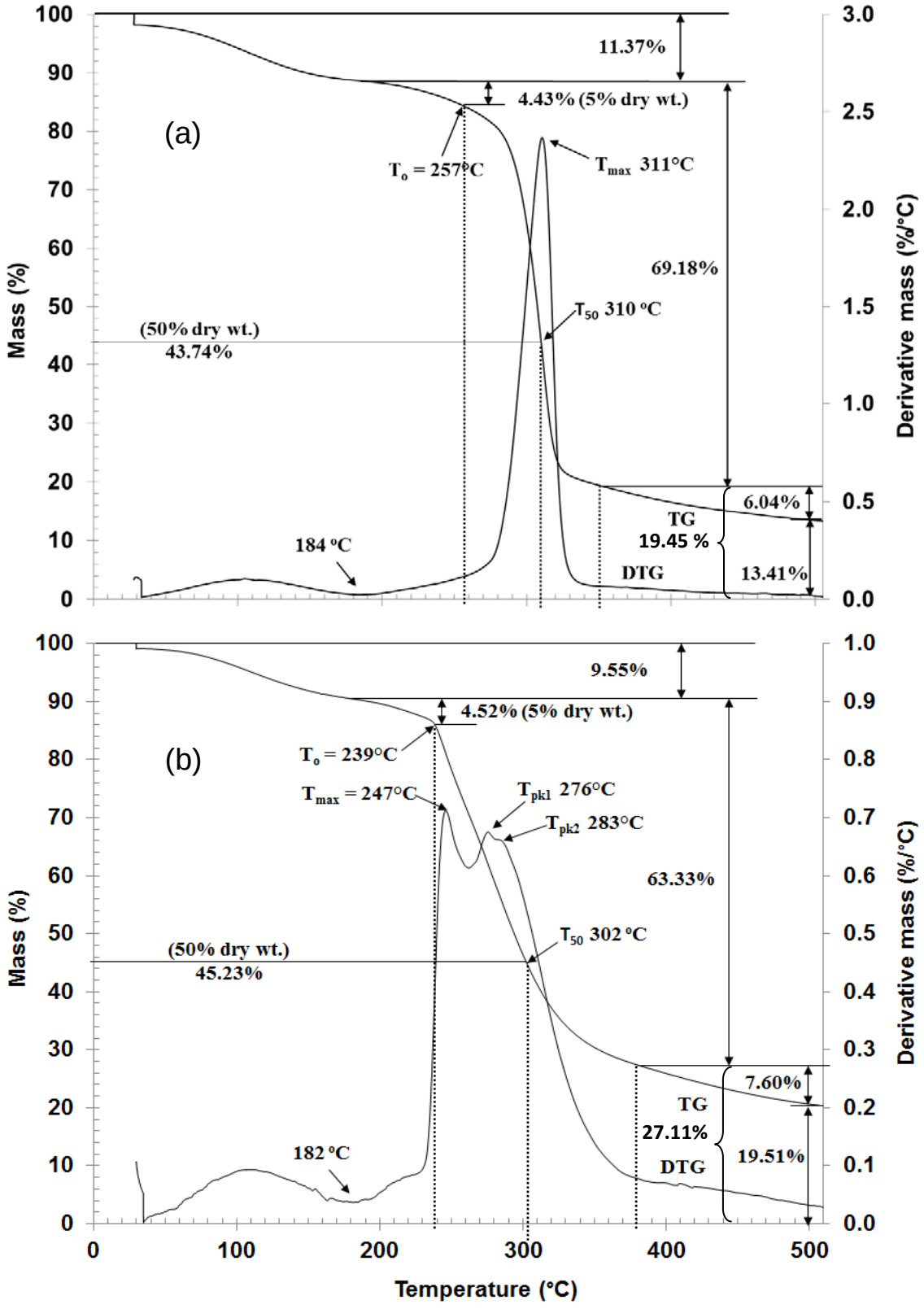

Fig. 3. **Thermal degradation curves for: (a) 0 wt% and (b) 10 wt% aconitic acid derived film.**

However, for 2 wt% AcA derived film T_o (258 °C) remained almost the same compared to the control T_o (257 °C) and T_{max} (315 °C) increased compared to the control T_{max} (311 °C). This may be explained by the effect of cross-linking between AcA and the starch polymers creating a more thermally stable film, which more than compensates for any thermal instability created by the small amount of residual AcA (Figure 3). In a similar way, Yu *et al.* (2005) demonstrated that thermal plasticised starch/glycerol film containing 1 and 3 wt% CA increased in the overall thermal degradation temperature (compared to the control). Yu *et al.* (2005) attributed the increased thermal stability to the formation of stronger hydrogen bonds between the CA and starch polymer chains.

There is an increased thermal stability for 0 to 15 wt% AcA derived films above temperatures of approximately 350 to 370 °C as indicated by a continual increase in residual degradation products from 19.5 to 29.5 wt%, respectively. The improved thermal stability at elevated temperatures may be attributable to the AcA derived film being cross-linked by AcA reacting with the starch polymer, in a similar way to that of CA derived starch based films (Ma *et al.*, 2009; Reddy & Yang, 2010; Shi *et al.*, 2007; Yu *et al.*, 2005). However the T_o and T_{50} temperature values for the 10 and 15 wt% AcA derived films are almost the same (within 2 °C) and are not significantly different having a similar overall thermal stability.

The T_o, T_{50} and T_{max} value decrease as AcA content for derived film increases from 5 to 15 wt%, indicates a decrease in thermal stability in that temperature range. This appears to contradict claims of improved thermal stability due to cross-linking (indicated for these films in the later part of this study) but may be explained by possible acid hydrolysis of starch polymer chains with increased AcA concentration (Yu *et al.*, 2005). The decreased thermal stability result is similar to the increased degradation between 220 and 320 °C for starch/glycerol film having increased CA content (Ma *et al.*, 2009; Reddy & Yang, 2010; Shi *et al.*, 2007).

Another trend noted is the increase in the amount of residue remaining after the major DTG degradation peak that increases from 19 wt% for the control to 24, 26, 27, and 29 wt% for 2, 5, 10 and 15 wt% AcA derived films, respectively. As well the remaining undegraded residue left over at 500 °C increases from 13 wt% for the control to 17, 19, 20 and 23 wt% for 2, 5, 10 and 15 wt% AcA derived films, respectively (Figure 3). The high temperature residual compounds resisting degradation may be compounds formed due to cross-linking of AcA in the starch/glycerol film. This same trend of thermally stable residue increase was noted for CA cross-linked starch/glycerol film and was attributed to CA crosslink formation (Reddy & Yang, 2010; Shi *et al.*, 2007; Yu *et al.*, 2005).

3.3 Tensile testing analysis

Tensile testing results show an increased softening of the film indicated by the decrease in the Young's modulus and tensile strength as well as the increase in strain (Table 3 and Fig. 4). As expected the 2 wt% AcA film was significantly stronger than all of the other AcA films. However, the Young's modulus for the 2 and 5 wt% AcA derived films were not significantly different indicating the films have similar stiffness. This may simply be due to the small difference in the AcA mixture content. In addition however the strain at yield and strain at break for the 0, 2 and 5 wt% AcA derived films were not significantly different. Likewise there were no significant differences between the Young's modulus or strength values between the 10 and 15 wt% films. However for 10 and 15 wt% films the strain at yield was 58 and 99 % and the strain at break was 64 and 102 % respectively, which demonstrated significantly increased tensile strain values, likely due to increased free AcA.

The significant different trends between the films are more easily highlighted by the strain vs stress graphs for the films (Figure 4). The tensile graph shows a steady decrease in tensile strength between 0, 2 and 5 wt% AcA derived films, then a significant difference

Table 3 Tensile analysis of aconitic acid derived film*

AcA (%)	Young's modulus (Mpa)	Max. Tensile Stress (Mpa)	Strain at yield (%)	Strain at break (%)
0	643a	8.80a	2.11c	2.89c
2	419b	5.62b	2.68c	8.40c
5	328b	3.76c	2.25c	5.97c
10	61.7c	1.18d	57.7b	63.7b
15	38.1c	1.25d	99.0a	101.7a

* Figures under each property with the same letters are not significantly different ($P < 0.05$).

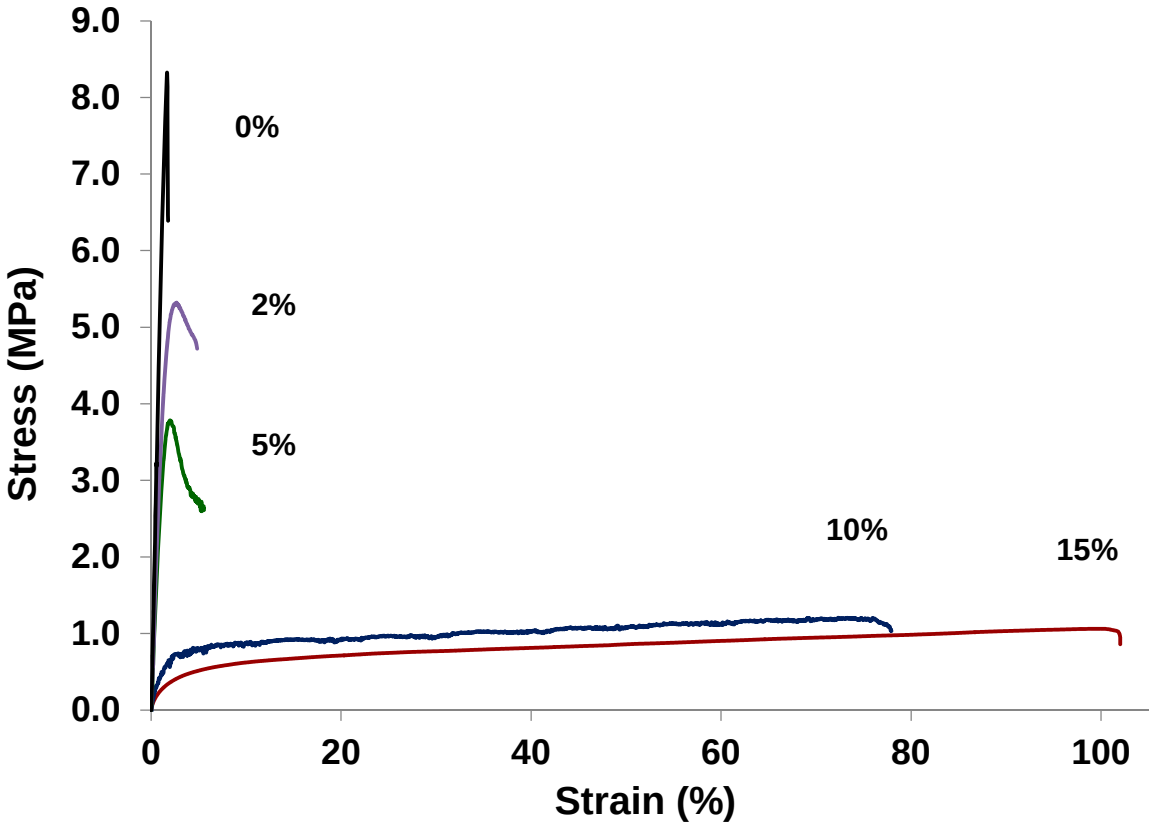

Fig. 4. Tensile stress vs strain curves for: 0, 2, 5, 10 and 15 wt% aconitic acid derived films.

between 10 and 15 wt% AcA derived films, a similar result to that for CA (Ghanbarzadeh *et al.*, 2011). The Young's modulus and tensile strength decrease by approximately 86 % and 67 %, respectively, while the strain at break and strain at yield increase by approximately 29 x and 13 x respectively.

A similar steady decrease in tensile strength was also found by Yu *et al.* (2005) who thermally extruded starch/glycerol film with (0.6, 1, 2, and 3 wt%) CA additions. The higher processing temperature for extrusion compared to that for the casting method explains why less CA had a similar result with the higher temperature increasing the CA reaction. However, Ghanbarzadeh *et al.* (2011) for solvent cast starch/glycerol film with 5 and 10 wt% CA added increased the tensile strength, whereas a dramatic decrease in tensile strength occurred while the CA was increased to 15 and 20 wt%. Further strength increases were achieved by the addition of carboxymethyl cellulose (CMC) fibre to the CA cross-linked starch-based film with strength increased from 6.6 MPa for film without CMC fibre to 16.1 MPa for film with 20 wt% CMC (Ghanbarzadeh *et al.*, 2011). Thermal extrusion using organic acid as a cross-linking agent also acted as a compatibiliser for starch/polyester (poly butylene adipate co-terephthalate) (PBAT) film to increase the strength from 4.1 MPa for the control to 6.7 and 6.8 MPa for (1.5 wt%) CA and TA films respectively (Olivato *et al.*, 2012).

Adjusting the glycerol content to optimise film strength as performed by Reedy & Yang (2010) was not explored due to time constraints. However, increasing the tensile strength by reducing the glycerol content may result in loss of the desirable elasticity property of the film. However, neither Ghanbarzadeh *et al.* (2011) nor Yu *et al.* (2005) working on CA films showed the dramatic increase in the elongation at yield and break as shown by the addition of 10 and 15 wt% AcA (Fig. 4).

3.4 DMTA analysis

DMTA results show that the addition of AcA made the film less rigid and more flexible compared to the control film as indicated by the decrease in E′ and E″ as well as the decrease in T_g (Table 4). This result supports the same general trend shown for tensile testing results. The similar E′ and E″ values for 2 and 5 wt% AcA derived films correlate with the similar Young's modulus values and indicate the films have similar stiffness. However the low T_g (49 °C) for the 2 wt% AcA derived film contradicts this since the 5 wt% AcA derived film has a higher T_g (63 °C) that indicates the 5 wt% AcA derived film is stiffer than the 2 wt% AcA derived film. The 10 wt% AcA derived film appears more rigid than either the 5 or 15 wt% derived AcA films since E′, E″ and T_g are significantly larger which appears to contradict the tensile results. The reason for the increased stiffness for 10 wt% AcA derived film (compared to 5 and 15 wt% AcA derived film) may be due to more cross-linking than the 5 wt% AcA derived film and having less plasticising residual AcA present compared to the 15 wt% AcA derived film. It would seem that there is a trade-off between the AcA plasticising effect to soften the film and the AcA cross-linking effect to stiffen the film.

Table 4 DMTA analysis of aconitic acid derived film*

Ac Acid (%)	E′ (MPa)	E″ (MPa)	T_g (°C)
0.0	1849a	84d	99a
2.0	519c	133b	49e
5.0	489d	136b	63c
10.0	814b	178a	71b
15.0	212e	95c	57d

* Figures under each property with the same letters are not significantly different ($P < 0.05$).

3.5 FTIR

The analysis of the FTIR spectra shows the typical spectra for starch/glycerol (control) film (Gilfillan *et al.*, 2014) and there was little change between the different films derived from different amounts of AcA. All spectra are normalised (so their peak heights could be compared) on the distinct 1150 cm^{-1} spectral peak which gave the least variation between films. However, the spectral peak for 0 wt% AcA derived film located at 1645 cm^{-1} showed a continued shift to 1635, 1627 and 1603 cm^{-1} for film derived with 2, 5 and 10 wt% AcA, respectively (Fig. 5). This trend is reversed for 15 wt% AcA derived film having a wavenumber value of 1627 cm^{-1} similar to the wavenumber for 5 wt% AcA derived film.

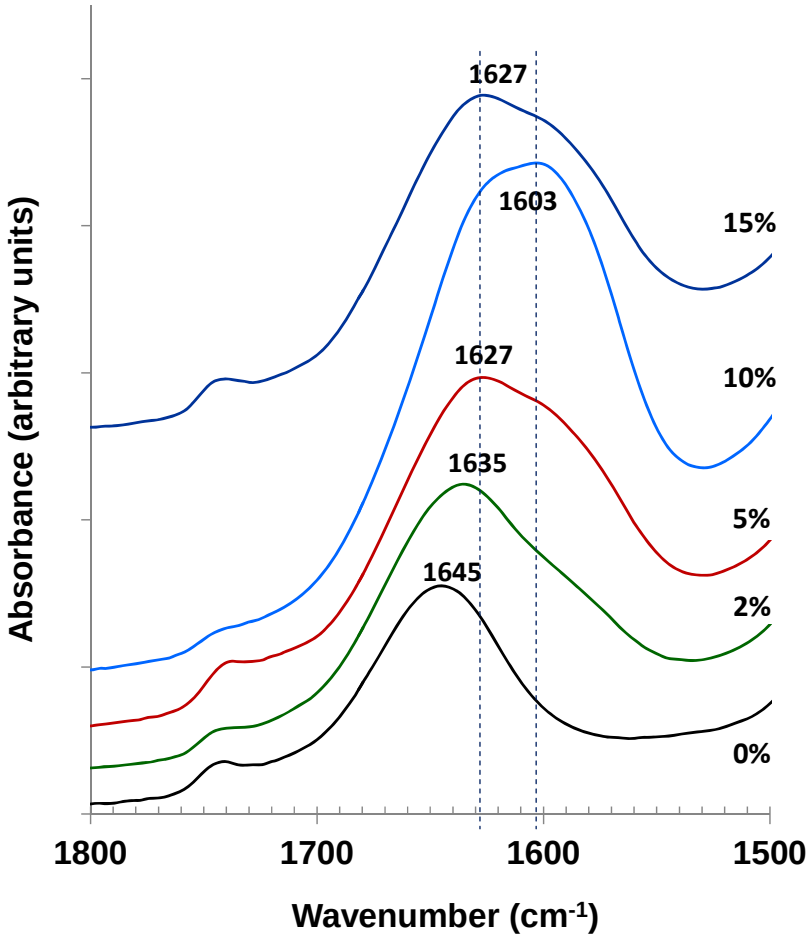

Fig. 5. FTIR spectra showing peak shifts of cast films derived from starch/glycerol mixtures that contain 0, 2, 5, 10 and 15 wt% AcA (starch wt% basis) from bottom to top (conditioned at 23 °C, 58% RH).

The FTIR peaks besides showing shifts in wavenumber are of higher intensity and broader than the control, indicating increased hydrogen bonding (Figure 5). Since the moisture in all the films is similar (Table 1) it may be that the increased hydrogen bonding of starch hydroxyl groups with the increased AcA free-residue present similarly to that for CA starch film (Yu *et al.*, 2005). This hydrogen bonding between the starch matrix and AcA also may be acting in a similar manner to an increased presence of water here.

3.6 Swelling and Solubility

Swelling and solubility studies are used to determine the degree of cross-linking in films (Radi *et al.*, 2010). The results (Fig. 6) show that for 0 wt% AcA derived film there was approximately 3x as much (74 wt%) of the film dissolved compared with 5 wt% AcA derived film (28 wt%) each film submerged in water (23 °C). These results are similar to the solubility results found by Reddy and Yang (2010) for (15 wt% glycerol) starch film cross-linked with 5 wt% CA having a 25 wt% solubility and the control 75 wt% solubility, after immersed in 50 °C water for 35 days. The solubility results were also similar to that of Ghanbarzadeh et al. (2011) for (40 wt% glycerol) starch-based film cross-linked with 10 wt% CA having 24 wt% solubility.

The trend (Fig. 6) shows that overall the 5 wt% AcA derived film was the least soluble which indicates it is the most cross-linked of all the films. However the 10 and 15 wt% AcA derived films also show similar solubility and therefore similar cross-linking.

The swelling ratio (Q) results (Fig. 6) closely follows the same trend as that for the solubility results. The exception however is that Q for the 2 wt% AcA film was unexpectedly the lowest value. This result may be due to excessively blotting out the moisture from the small amounts of gel left from hydration. If excessive water was removed then the swelling value would be inadvertently reduced. The swelling data for 5, 10 and 15 wt% AcA derived

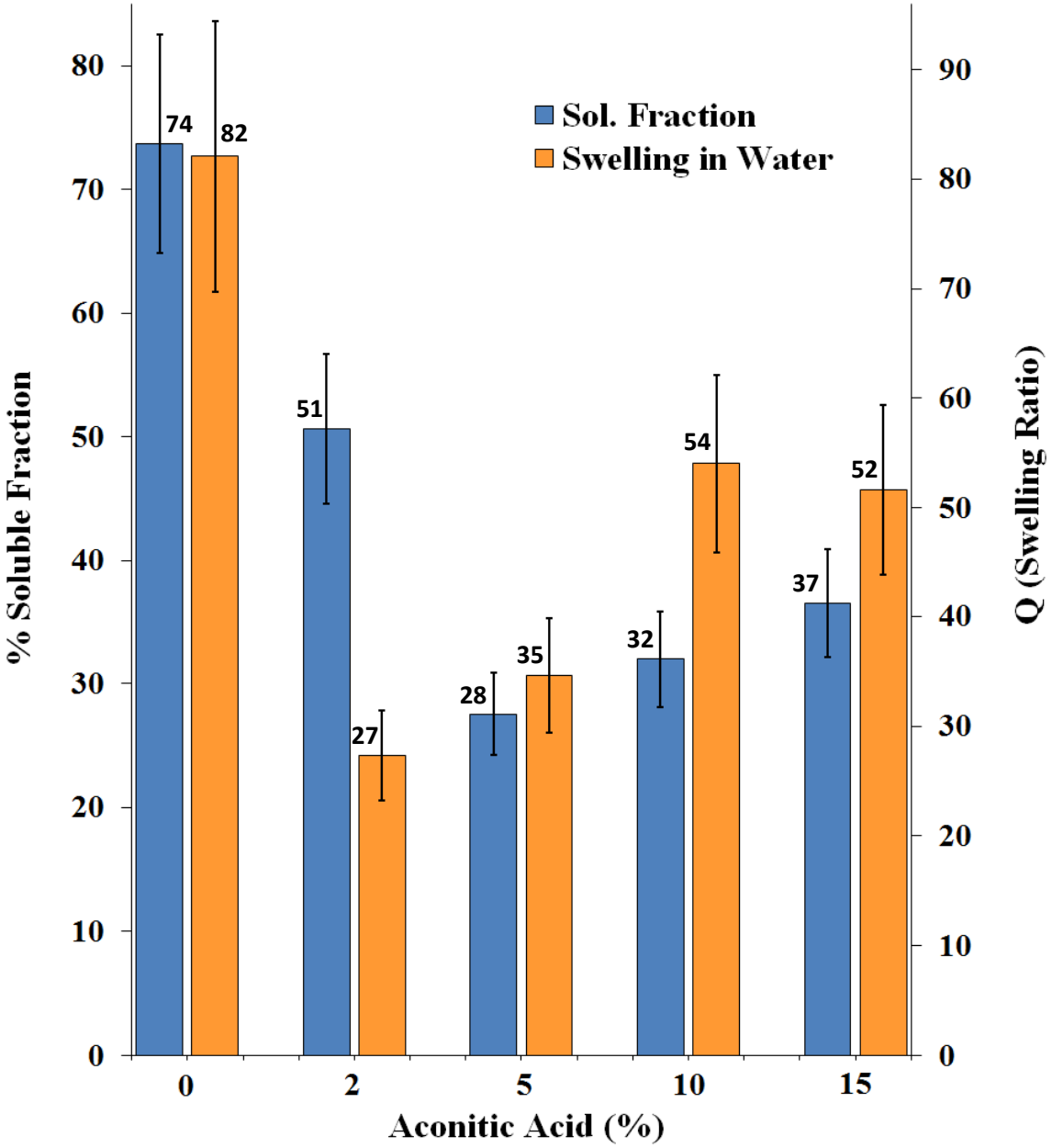

Fig. 6. Solubility and swelling derived from starch/glycerol mixtures that contain 0, 2, 5, 10 and 15 wt% aconitic acid (starch wt% basis).

film was considered more representative since larger amounts of gel present reduced the magnitude in the moisture error for excess blotting of the sample. Besides, the solubility data was considered more reliable since this was based on the dry weights of the soluble portion and insoluble gel. Overall the 0 wt% AcA derived film (Q = 82 times) swelled approximately

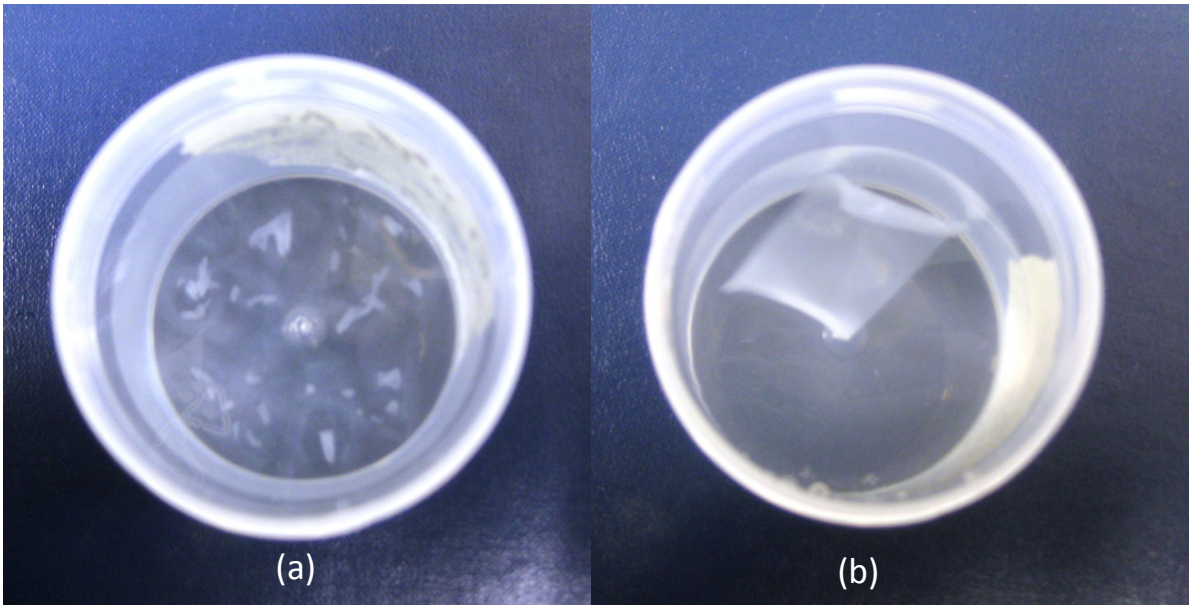

Fig. 7. Top view of solubility/swelling test of cast films derived from starch/glycerol mixtures containing (a) 0 wt% and (b) 15 wt% aconitic acid (based on starch wt) after four days being immersed in pure distilled water (23 °C).

2.5 times more than the 5 wt% AcA derived film (Q = 35 times). Therefore, it was considered that the 5 wt% AcA derived film overall was the most cross-linked film based on both the solubility and swelling data after consideration of the experimental error.

The difference in solubility between the films is easily seen when viewed in the solution after solubility/swelling equilibrium was reached in 4 days. The 0 wt% AcA derived film was mostly disintegrated (similar to the 2 wt% AcA derived film) and mostly dissolved whereas the 15 wt% AcA derived film (similar to the 5 and 10 wt% AcA derived film) is seen still to be in tact (Fig. 7).

3.7 Average molecular weight between cross-links (M_c)

The swelling data (Fig. 6) is used as the basis for determining the average molecular weight between cross-linked polymer network structures (M_c) values (Table 5) based on the Flory-Rehner rubber elasticity model (Elbert *et al.*, 2001; Flory, 1953; Flory & Rehner, 1943; Radi *et al.*, 2010). The low M_c for 2 wt% AcA derived film was considered lower than the

actual value due to experimental error and this is backed by more accurate solubility data based on dry weight measurement. The calculated M_c value for 0 wt% AcA film, 465.2 x 10^3 (g/mole) is approximately 2.4 times larger than that for the most cross-linked 5 wt% AcA film, 197.4 x 10^3 (g/mole). The smaller M_c value indicates that there is much more cross-linking in the 5 wt% AcA derived film, since a lower M_c indicates greater cross-linking (Radi *et al.*, 2010). The M_c result is as expected being derived from the swelling volume fraction that was approximately 2.5 times less for the 5 wt% AcA derived film compared with the 0 wt% AcA derived film.

Table 5 Molecular weight between two cross-links M_c (calculated)

AcA (%)	M_c* calculated (g/mole) x 10^3
0	465.2
2	123.9
5	197.4
10	353.0
15	339.1

*based on swelling data

4. CONCLUSIONS

Starch-based cast films produced from solutions containing AcA were effectively cross-linked at relatively low AcA concentrations (2-5 wt%) with the films having a lower water solubility and decreased swelling coefficient. At higher concentrations AcA acted as a strong plasticising agent which impacted on the elastomeric and tensile film properties by increasing the elongation at break, reducing the tensile strength and softening the film. However the films decreased in thermal stability with increased AcA content. Optimisation of the elastomeric properties of these films may lead to novel applications both in the food and packaging industries. In medicine, a potential use may be as a wound 'barrier film' dressing if combined with anti-bacterial agents. As food packaging material the AcA film may be used directly to prevent oxidation since AcA inherently has anti-oxidizing properties.

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